Amendment A

Paper No. 3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

Jan Vijg

Serial No.: 09/306,333 Group Art Unit: 1643

Filed: May 6, 1999 Examiner: Squaya, J.

For: BRCA1 and hMLH1 Gene Primer Sequences And Method For Testing

#5/B 2/7/01

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Hon. Commissioner of Patents
and Trademarks
Washington, DC 20231

Sir:

Replying to the Office communication of May 16, 2000, please amend the application as follows:

Commencing with the first page containing the title, please number the successive pages of the specification 1 through 12, respectively.

Please substitute for pages 6-12 of the specification, as above numbered, the following pages 6-11 identified as Tables 1 through Table 4:

In the claims, please delete claims 1-3, and substitute the following:

--10. A method for detecting mutations in the BRA1 and hMLH1 genes comprising providing PCR primers capable of amplifying the entire coding sequence of the BRCA1 and hMLH1 genes, amplifying a test sample containing nucleotide sequences by PCR with these primers producing a first set of amplification products; subjecting this first set of amplification products to short distance multiplex PCR; providing a second set of amplification products using substantially the respective primer pairs of Tables 4 and 1 for this short distance PCR; and subjecting the second set of amplification products to two-dimensional gel electrophoresis to produce a characteristic spot pattern for a specific mutation in either the BRCA1 or the hMLH1 gene.--

--11. The method of claim 10 wherein non-denaturing gels and buffer materials are used so as to enable combined mixtures of multiple groups of BRCA1 ~~and hMLH1~~ genes to be subjected to the electrophoresis together.

Please rewrite claim 4 as follows:

~~4.~~ (Amended) Test kits for enabling BRCA1 [and hMLH1] gene testing [prepared by the method of claim 3] comprising the short PCR primer pairs listed in Table 4 mixed in about 20mM of Tris-HCl, 50mM KCl, 25µM of d NTP and 5% formamide.

--5. The test kits of claim 4 wherein a gel or gel material is also provided comprising acrylamide and bisacrylamide in a ratio of about 37.5 to 1, in a buffer. --

--6. The test kits of claim 5 wherein the gel or gel material is provided with about 20-65% of UF (urea and formamide). --

--7. Test kits for enabling hMLHl gene testing comprising the short PCR primer pairs listed in Table 3 mixed in about 20mM of Tris-HCl, 50mM KCL, 25µM of d NTP and 5% formamide. --

--8. The test kits of claim 7 wherein a gel is also provided comprising acrylamide and bisacrylamide in a ratio of about 37.5 to 1, in a buffer. --

--9. The test kits of claim 8 wherein the gel is provided with about 25-75% of UF (urea and formamide).--

Please insert before the claims, the "SEQUENCE LISTING" appended to the end of this amendment.

REMARKS

Claims 1 through 4 have been rejected under 35 USC Sec. 112 as indefinite.

As re-written herein and as later more fully explained, these objections have been obviated in the substitute method claims 10 through 11, amended kit claim 4, and additional kit claims 5 through 9.

Titles and Tables have also been provided to conform to the requirements of U.S. Patent Office practice, and the specification has been checked for grammatical errors.

As required by the Office, the applicant has revised the sequence listings on pages 6, on, to comply with the requirement and format of the rules, and without new matter--such being appended to the end of this amendment.

Specifically, new "Table 1- Primer Sequences And Target Fragment Length For hMLH1 Long Multiplex PCR", replaces page 6 that was originally entitled "6, The primer sequences for long and short PCR for the MLH1" (erroneously stated as "BRCA1"). Table 1 starts with the same Exons 1-4 (length or product size 10.8kb) and ends with Exons 14-19 (10.5kb), referring to precisely the same sequences originally listed, but by reference to the required SEQ. ID Nos. now provided on the new "SEQUENCE LISTING".

Table 2 starts with the primer sequences and target fragment length for the BRCA1 long multiplex PCR listed on original page 9, referring to the very same sequences originally listed, but now by reference to SEQ. ID Nos. provided in the new "SEQUENCE LISTING". These are presented starting with BRCA1 1-3F and 1-3R (9.9kb) and ending with 11.4kb BRCA1 21-24F and 21-24R, just as on original page 9.

Table 3 presents precisely the primer sequences clamping sequences, target length or size, and melting temperatures for hMLH1 short multiplex PCR and two-dimensional electrophoresis, as presented at the bottom of original page 6 ("B. Primer Pairs for Short PCR") and on original page 7: from Exon fragment 12.1 through 19.1, and again now referencing the SEQ. ID Nos. in the "SEQUENCE LISTING"--all identical to the sequences information originally presented on pages 7 and 8 (clamping sequences). .

Table 4 (three sheets) similarly lists the same data for Exon Fragments 2 through 10 and 11.1 through 24, originally listed on pages 10, 11 and 12 for BRCA1 short multiplex PCR and two-dimensional electrophoresis.

Prior Art Rejection

The claims have further been rejected under USC Sec. 103 as the obvious extension to BRCA1 and hMLH1 genes of the DNA - PCR amplification prior teachings of the applicant Vijg-WO96/39534 and US Patent 6,007,231, and in view of ancillary teachings of Patent 5,948,697 to Park et al and 5,822,855 to Liskay et al.

The Office appreciates, however, that while the Liskay et al and Park et al patents disclose "mutations in the hMLH1 and BRCA1 genes and their link to cancer", actually "Vijg does not teach testing gene sequences of the BRCA1 and hMLH1 gene". While rejecting the claims on this combination of prior art, therefore, the Office has suggested that if applicant provides a

> "showing of unexpected results in using the primers of the disclosed invention which gave better results (in the claimed method) than other primers also generated based on the teachings and methods of Vijg and Vijg II,"

this

> "could aid applicant in overcoming the rejection made under 35 USC 103(a)."

Specifically, the Examiner notes that

> "a showing that the claimed primers gave better results, which were not expected given the teaching of Vijg on how to design primers based on a known sequence, could overcome the obviousness rejection."

Through the accompanying Declaration of Dr. Vijg's associate, R. David Rines, such vastly better and unexpected results with BRCA1 and hMLH1 gene sequencing have indeed been effected with the different primer sequences and designs of the present application, as pointed out in the Declaration. It is believed, accordingly, that this showing satisfies the Examiner's requirement for overcoming the obviousness rejection.

Specifically, following the Vijg teachings in connection with the scanning of the RB1 genes as described in the Vijg patent 6,007,231 and his earlier patent 5,814,491 (Fig. 5), whole exon coverage is effected in a single fragment (for example, 10, 11, etc.). It

has been discovered in connection with the present invention that to attain high resolution, clarity and reliability of assays, the sequence of BRCA1 genes requires a substantial number of splitting of the genes into fragments--indeed, in connection with exon 11, fifteen fragments labeled 11.1 through 11.15 in Fig. 1B of the present application; and on original page 10 of the specification, re-presented as new Table 4. In addition, while the prior RB gene scanning used clamps, it was necessary for both the BRCA1 and hMLH1 genes to depart from the prior Vijg concept of a single standard clamp bar primer pair and to provide instead clamps of variable sequence and links to induce a stable melting domain.

Specifically, in connection with the hMLH1, fragments 12.2, clamps 8 and 40; and fragment 14, clamps 45 and 5, as listed on original page 7 of the specification, and now in Table 3. Similarly, in connection with the BRCA1 scanning, pairs of clamping units were required, as at 11.1, 11.2, 11.4, etc., page 10 of the original specification (now Table 4), to induce such a stable melting domain.

In claiming the primer pair sequences and clamps of Tables 2 and 4, not taught in these prior Vijg references, new method claim 10 (adopting the language suggested by the Office on page 5 of its action), defines over the earlier sequence requirements of Dr. Vijg as detailed in the Rines declaration, enabling the attainment of the novel highly improved resolution and clarity shown in Fig. 1A for the hMLH1 gene, and in Fig. 1B for the BRCA1 gene.

Method claim 11 depends from claim 10 adding combined mixture electrophoresis.

Test kit claim 4 has been amended to recite the specific novel BRCA1 kit primer pairs and ingredients; and dependent claims 5 and 6, the preferred ratio of acrylamide and bisacrylamide gels and preferred range of UF, respectively.

Claims 7-9 are similar to claims 4-6 but are directed to the novel primer pairs and ingredients for the hMLH1 gene.

All of claims 4-11 appear thus clearly to define the unexpected results outlined in the Rines declaration with the specific novel primers of the invention, not disclosed

in any of the Vijg references, taken alone or in any combination with the other references.

Applicant also attaches the required disc on which the copy in computer readable form is the same as the sequencing testing, which has also strictly followed the requirements of Sec. 1.821-5.

Any costs incurred by this filing, including for any required extension(s) of time, petition for which is hereby made, may be charged to account No. 18-1425 of the undersigned attorneys.

Respectfully submitted,

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